Future Development of Lymphoproliferative Disorders in Patients with Autoimmune Hemolytic Anemia

Sabah Sallah,1 Jim Y. Wan, and L. Robert Hanrahan
Divisions of Hematology/Oncology [S. S.] and Biostatistics [J. Y. W.], University of Tennessee Health Science Center, Memphis, Tennessee 38103, and Department of Pathology and Laboratory Medicine, East Carolina University, Greenville, North Carolina 27858-4353 [L. R. H.]

ABSTRACT
The association between autoimmune hemolytic anemia (AIHA) and subsequent appearance of lymphoproliferative disorders (LPDs) has not been properly addressed in large-scale studies. We evaluated 107 patients with idiopathic (67 patients) or underlying (40 patients) immune disorders diagnosed with AIHA between 1992 and 1999. The following variables were examined in univariate and multivariate analysis: age; sex; type of AIHA (warm- or cold-active antibodies); presence of underlying immune disorders; and serum monoclonal protein. Of the 107 patients, 19 (18%) developed malignant LPDs. The median time to develop malignancy was 26.5 months (range, 9–76 months). At multivariate analysis, advanced age (P = 0.005), underlying autoimmune diseases (P = 0.002), and the presence of serum gammopathy (P = 0.045) were risk factors for future development of LPDs in these patients. Also, serum monoclonal IgM protein was a significant predictor (P = 0.0001) for the appearance of LPDs in patients with AIHA. The present study provides evidence that AIHA in some patients should be considered as a precursor of malignant LPDs. Knowledge of certain characteristics may help identify patients at risk for this transformation; periodic clinical and laboratory assessment of these patients is warranted.

INTRODUCTION
AIHA is caused by autoantibodies against antigens on the surface of RBCs. The result is increased destruction and shortened life span of erythrocytes. Serologically, AIHAs are classified according to the temperature of reaction into warm and cold (1). Warm AIHA is usually caused by IgG autoantibodies reactive at 37°C, whereas IgM cold antibodies react optimally at lower temperatures but may show significant hemolysis at 37°C. Occasionally, immune hemolysis may be caused by mixed warm and cold antibodies. Another clinically distinct but uncommon type of hemolytic anemia is caused by cold-active IgG antibodies and occurs through activation of the C5-C9 membrane attack complex (1). This entity is referred to as paroxysmal cold hemoglobinuria and is characterized by hemoglobinuria after exposure to cold (1).

There is a well-described association between LPDs and AIHA (2, 3). It has been estimated recently that 4.3% of patients with CLL develop AIHA (4). Such occurrence does not appear to have a negative impact on the survival of patients with CLL. Unfortunately, AIHA occurring in other LPDs such as NHLs has not been investigated in large-scale studies (5). Similarly, the reverse association or the subsequent development of LPDs in patients with AIHA and no apparent malignancy has been rarely addressed outside of scattered case studies (6).

To further clarify the association between immune hemolysis and future appearance of LPDs, we evaluated a cohort of patients with AIHA and no underlying malignancy at the time of diagnosis. The purpose was to determine the presence of certain characteristics or risk factors that might predict malignant transformation.

MATERIALS AND METHODS
Data on patients with AIHA diagnosed between 1992 and 1999 in three major medical centers (University of Tennessee, Memphis, TN; University of North Carolina, Chapel Hill, NC; and East Carolina University, Greenville, NC) were evaluated. Patients were identified through referral to hematology services of the participating institutions. The clinical and laboratory data of these patients were obtained by a process of chart review. The criteria for inclusion were a positive direct antiglobulin test and evidence of hemolysis that included reticulocytosis, increased indirect bilirubin, increased lactate dehydrogenase, and decreased haptoglobin. Patients were excluded from the analysis if they had any of the following conditions: malignancy; positive test for HIV; recent infection; and drug-induced hemolysis. Patients with malignant conditions at the time of diagnosis of AIHA were excluded on the basis of history and routine clinical and laboratory assessment. Bone marrow aspiration and biopsy were not performed in the absence of clear indication from peripheral blood film (i.e., bi- or pancytopenia, immature cells). A total of 107 patients met the above criteria. Of the 67 patients excluded from the study, there were 29 with underlying malignancy (mainly CLL), 17 patients with infections (mainly HIV), and in 14 patients the hemolysis was thought to be drug related. Seven patients were excluded because of lack of appropriate follow-up.

Treatment of AIHA included corticosteroids in 93 patients,
immunosuppressive agents, such as cyclophosphamide alone or in combination with prednisone and/or vincristine; cyclophosphamide and azathioprine were used in 27 patients. i.v. immunoglobulin was used in 9 patients, and plasmapheresis was used in 3 patients. Splenectomy for refractory warm AIHA was performed in 12 patients with warm and in 2 patients with mixed AIHA.

**Statistical Consideration.** The two groups (with and without LPDs) were compared using two-sample t test or χ² test in univariate analysis. Logistic regression was then used to determine the simultaneous effects of these variables. ORs and 95% CIs were reported. Fisher’s exact test was used to compare the type of monoclonal gammopathy, and Wilcoxon test was used to compare the size of monoclonal protein between the two groups. Variables with \( P < 0.05 \) were declared significant.

**RESULTS**

The median age of the 37 men and 70 women included in the current investigation was 54 years (range, 23–83 years). The median age for the patients with warm and cold AIHA was 49 and 67 years, respectively; \( (P = 0.0001) \). Table 1 shows the characteristics of all patients in this analysis. There were 78 patients (72.9%) with warm AIHA, 22 patients (20.5%) were diagnosed with cold AIHA, and 5 patients were diagnosed with mixed AIHA. There was no statistically significant difference in the number of patients with underlying autoimmune disorders between the patients with warm and cold AIHA (33 versus 7; \( P = 0.429 \)). Of the 107 patients, 19 (18%) developed malignant lymphadenopathy, within a median of 26.5 months (range, 9–76 months). Of these, 5 had CLL, 1 Hodgkin’s disease, 1 angioimmunoblastic lymphadenopathy with dysproteinemia, 1 Waldenstrom’s macroglobulinemia, and 11 patients were diagnosed with NHL. The breakdown for the patients who developed NHL was 6 follicular center, 1 small lymphocytic, 1 immunoblastic, 2 peripheral T-cell, and 1 cutaneous T-cell lymphoma. Among the patients who developed LPDs, 2 had rheumatoid arthritis, 1 temporal arteritis, 1 Crohn’s disease, 1 systemic lupus erythematosus, 1 Sjogren’s syndrome, and 1 patient had thyroiditis.

Table 2 depicts the results in univariate and multivariate analysis. According to univariate analysis, advanced age (\( P = 0.0007 \)), cold AIHA (\( P = 0.003 \)), and serum monoclonal gammopathy (\( P = 0.003 \)) were parameters that more likely to be present in patients who developed LPDs. In multivariate analysis, older patients with AIHA were more likely to develop LPDs than younger individuals (\( P = 0.005; OR, 1.105; 95\% CI, 1.02–1.18 \)). Patients with underlying autoimmune diseases were also more likely to manifest LPDs during the duration of follow-up (\( P = 0.002; OR, 14.1; 95\% CI, 2.71–75.1 \)). A significantly larger proportion of patients who developed LPDs had monoclonal gammopathy when compared with patients who did not develop malignancy (\( P = 0.045; OR, 8.56; 95\% CI, 1.04–70.19 \)).

Of the 19 patients who developed LPDs, 4 (21%) had IgM and 1 patient had IgG. The type of monoclonal gammopathy in all 5 patients (6%) without LPDs was IgG. Serum monoclonal IgM protein was more likely to be present in patients with subsequent malignant LPDs (\( P = 0.0001 \)). There was no statistically significant difference in the size of serum gammopathy between the patients who developed LPDs (median, 1.8 g/dl) and those without LPDs (median, 1.8 g/dl; \( P = 0.761 \)).

Of the 27 patients who received immunosuppressants, 4 developed lymphoid malignancies, whereas there was no confirmed history of exposure to such agents in the other 15 patients who developed LPDs (\( P = 1 \)). Among the 19 patients who developed LPDs, there were 3 with a history of prior splenectomy (\( P = 0.135 \)).

**DISCUSSION**

There is well-described association between CLL and warm or cold autoimmune hemolytic anemia (2–4). The vast majority of the patients with CLL and hemolysis have polyclonal antibodies not originating from the malignant clone (2, 3, 7, 8). For example, polyclonal IgG antibodies are found that may be the result of dysregulation of polyclonal CD5⁺ B cells by T helper cells activated by the malignant CD5⁺ B cells (7, 8). A warm antibody AIHA may result. Occasionally, however, cold AIHA may result from IgM antibodies directed against the I/β blood group antigen system. These monoclonal cold agglutinations are likely to be produced by the malignant CD5⁺ B cells (9).

The pathogenesis of the reverse, or LPDs, developing after a diagnosis of AIHA is largely unknown. One possibility is the failure to recognize a developing lymphoproliferative process in its early stages and the expression of autoreactive clones. More likely, the development of a lymphoma is a multistep process and a proliferative phase involving chronic antigenic stimulation or viral infection may presage the mutation, giving the clone autonomous growth. Additionally, defects in the Fas/Fas ligand or the antiapoptotic protein Bcl-2 or the loss of certain homeostatic mechanisms that prevent the overexpansion of autoreactive clones in some patients with AIHA may lead to subsequent development of lymphoid malignancies (10). Such apoptotic

---

**Table 1** Characteristics of 107 patients with AIHA

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Number</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>54</td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>(23–83)</td>
<td></td>
</tr>
<tr>
<td><strong>Sex</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>78</td>
<td>72.9%</td>
</tr>
<tr>
<td>Female</td>
<td>29</td>
<td>27.1%</td>
</tr>
<tr>
<td><strong>AIHA</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Warm-active antibodies</td>
<td>78</td>
<td>72.9%</td>
</tr>
<tr>
<td>Cold-active antibodies</td>
<td>22</td>
<td>20.5%</td>
</tr>
<tr>
<td>Mixed</td>
<td>5</td>
<td>5.5%</td>
</tr>
<tr>
<td>Paroxysmal cold hemoglobinuria</td>
<td>2</td>
<td>2.3%</td>
</tr>
<tr>
<td>Serum monoclonal gammopathy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IgG</td>
<td>6</td>
<td>5.6%</td>
</tr>
<tr>
<td>IgM</td>
<td>5</td>
<td>4.6%</td>
</tr>
<tr>
<td>No. of patients with underlying immune disorders</td>
<td>40</td>
<td>37%</td>
</tr>
<tr>
<td>No. of patients who developed malignancy</td>
<td>18</td>
<td>17%</td>
</tr>
<tr>
<td>Median time to develop malignancy (mo)</td>
<td>26.5</td>
<td></td>
</tr>
<tr>
<td>Follow-up (mo)</td>
<td>36</td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>6–76</td>
<td></td>
</tr>
</tbody>
</table>
defects have been described in patients with the autoimmune lymphoproliferative syndrome. It is well known that patients with this syndrome are at very high risk of developing malignant LPDs during their lifetime (11–13).

In the current analysis, we evaluated the relevance of certain clinical and laboratory features in the occurrence of LPDs in patients with immune hemolysis. At multivariate analysis, older age, presence of underlying immune diseases, and serum monoclonal protein were independent factors significantly correlated with the appearance of malignant disorders. The progressive decline in lymphocyte function and self-tolerance associated with the aging process may be an explanation for advanced age being an independent predictor of future malignant transformation (14). Coexistence of autoimmune disorders in some patients who developed LPDs is suggestive of dysregulation in the immune system and consistent with the known strong association between these disorders and lymphomas. Another variable that proved to be an important predictor of subsequent appearance of NHLs in this study is serum paraprotein. It has well been established that monoclonal gammopathy, especially the IgM type, is a precursor for developing multiple myeloma and other LPDs in some patients with no overt malignancy at the time of diagnosis (15). The presence of monoclonal gammopathy is likely an indicator of a defect in normal control mechanisms mediating the immune response and the existence of clonal or subclonal lymphocytes with malignant potential. It is interesting to note that cold AIHA is very predictive of the development of LPDs in the univariate analysis but is no longer significant in multivariate analysis. That is, cold AIHA by itself is a predictor but not in the presence of other factors. A detailed analysis (see “Results”) shows that patients with cold AIHA tend to be older, and the older patients tend to develop LPDs. Hence, when age is adjusted for in multivariate analysis, cold AIHA is not significant.

It is well known that agents such as cyclophosphamide and other immunosuppressants may be responsible for inducing secondary malignancies including myeloid and lymphoid disorders (16). The results from other series did not demonstrate statistically significant impact of these agents on the future appearance of lymphoid malignancies. However, it should be emphasized that more than one-third of the patients in this study had underlying autoimmune diseases, and immunosuppression was used on an intermittent or continuous basis to manage immune phenomena in these patients. It is possible that immunosuppressants potentiate the risk of LPDs in a subset of patients with AIHA and autoimmune disorders. This issue may be resolved by future trials that assess the role of immunosuppression on the appearance of malignant tumors in a homogeneous population of patients (patients with idiopathic AIHA). Similarly, few investigators have reported splenectomy as a possible risk factor for subsequent hematological malignancies (17, 18). Our analysis, however, did not support the role of splenectomy as predictor of LPDs in patients with immune hemolytic anemia.

We recognize that a major drawback of our study is the retrospective design. This certainly could have biased the selection of patients and subsequently influenced the follow-up and exaggerated the final results. On the other hand, it is also possible that a certain proportion of the patients with secondary AIHA caused by infectious agents may evolve into LPDs if followed for long periods. To our knowledge, the natural history of some patients with AIHA caused, for example, by HIV or EBV is not well described. One wonders if AIHA is an early manifestation of an undiagnosed LPD that was triggered by an earlier viral infection. The overlap is clear; unrecognized or unrecognized or subclinical dysfunction in the immune system may predispose to viral infections, AIHA, and LPDs. This remains an assumption but emphasizes the need for further investigation. Prospective, better designed studies may help resolve some of the unanswered questions in this field.

In conclusion, this study provides evidence and establishes an association between AIHA and future appearance of hematological malignancies. Knowledge of certain clinical and laboratory features present at the time of diagnosis may help identify a subset of patients at high risk for developing LPDs. Periodic clinical and laboratory evaluation of these patients, perhaps every 3 months, may prove to be effective in the early
detection of these malignancies. Whether this approach would eventually improve the outcome of these patients remains to be seen. Serial evaluations, however, are likely to provide insights on the natural history of both AIHA and the lymphoid malignancies developing in this setting. The relation between AIHA and the future appearance of a LPD is reminiscent of the association between monoclonal gammopathy of undetermined significance and subsequent development of plasma cell disorders. In our view, indefinite follow-up is warranted in both cases.

REFERENCES

Future Development of Lymphoproliferative Disorders in Patients with Autoimmune Hemolytic Anemia

Sabah Sallah, Jim Y. Wan and L. Robert Hanrahan